Laminar Distribution of $GABA_A$ Receptor α_1, β_2, and γ_2 Subunit mRNAs in the Granular and Agranular Frontal Cortex of the Rat during Pre- and Postnatal Development

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The expression of mRNAs encoding the three $GABA_A$ receptor subunits that are associated with the most abundant benzodiazepine-sensitive $GABA_A$ receptor in adult cortex, that is, the α_1, β_2, and γ_2 subunits, was studied in rat cortex during pre- and postnatal development by means of *in situ* hybridization. γ_2 and β_2 mRNAs become detectable in neocortex at gestational day 16 (GD16), α_1 at GD18. γ_2 mRNA exhibits the highest level of expression at early ages, while α_1 mRNA levels are low. β_2 mRNA rises steeply during the last days of gestation. Around birth, it shows the highest expression of the three subunits studied in cortex, and increases further until postnatal day 15 (PD15). The expression of α_1 subunit mRNA also increases markedly shortly before birth and accelerates between PD8 and PD15, when it reaches higher levels than the other two subunits. Following the initial high expression, γ_2 mRNA increases gradually and slowly until PD25.

During prenatal development, highest expression of all three subunit mRNAs is found in the upper layers of cortex, that is, cortical plate and marginal zone. The subplate layer does not start to express $GABA_A$ receptor subunit mRNAs until GD18. At birth, all developing layers of the cortex express mRNAs for the three subunits, except the marginal zone. Highest levels are found in the upper part of the cortical plate. At the end of the first postnatal week (PD8), the laminar distribution of mRNA expression in neocortex becomes more differentiated. For all three subunit mRNAs, highest expression is then observed in neuron-like cells in layer IV in the granular areas, and over layers III and upper V in agranular areas. Subsequently, between PD8 and PD25, increasing levels of expression are observed over the pyramidal cell layer V. This regionally differentiated, developmental pattern suggests a close relationship between development of $GABA_A$ receptor subunits, ingrowth of thalamocortical projections, and maturation of neocortical circuitry.

GABA (γ-aminobutyric acid) is a major inhibitory neurotransmitter in the neocortex (Krnjevic and Schwartz, 1966). The effects of GABA are mediated by two types of GABA receptors, that is, $GABA_A$ and $GABA_B$ (Bowery et al., 1987). The $GABA_A$ receptor is linked to a chloride channel and sensitive to benzodiazepines (Olsen and Tobin, 1990; Seeburg et al., 1990). It is a pentamere consisting of three different subunits, from five families, namely, α, β, γ, δ, and ρ subunits, with several subtypes of each subunit. The composition of the subunits determines the pharmacological and physiological properties of the receptor (Seeburg et al., 1990; Verdoorn et al., 1990; Knoflach et al., 1992). Recently it was demonstrated that a typical benzodiazepine (BZD)-sensitive $GABA_A$ receptor is composed of a combination of $\alpha_1\beta_2\gamma_2$ subunits (Sigel et al., 1990; Verdoorn et al., 1990). These three subunits, whose distribution patterns show a high degree of overlap, are abundantly present in the cortex of the adult rat (Malherbe et al., 1990; Seeburg et al., 1990).

During early prenatal development, GABA is found in cells and cellular processes that are present in all layers of the cortical anlage, especially in the marginal zone and subplate layers (Lauder et al., 1987; Van Eden et al., 1989; Meinecke and Rakic, 1992; Parnavelas, 1992). The neurons in these layers form a transient fetal network, which is important in the formation of connections between the cortex and distant brain regions and in the differentiation of neocortical structures (Shatz et al., 1988; Meinecke and Rakic, 1992). *In vitro* experiments have identified GABA as a trophic factor (e.g., Spoerri, 1987), and several authors have inferred an important developmental role for GABA in modulating cell differentiation and maturation *in vivo*. Furthermore, BDZ binding sites are present early, and these compounds have been shown to have a pronounced effect on brain development (Schlumpf et al., 1983, 1992b). Since most of the GABA signal transduction in the cortex implicates the $GABA_A$ receptor, and because of the pronounced effects of BZD on brain development, we have studied the presence of mRNAs for the α_1, β_2, and γ_2 subunits of the $GABA_A$ receptor in the frontal cortex during different stages of pre- and postnatal development.

The present article focuses on the detailed areal and laminar distribution of those three subunits that are known to be present in the common $GABA_A$ receptor with BDZ type I binding properties within the frontal cortex during pre- and postnatal development.

The prenatal distribution patterns of these subunits in the CNS were presented in a preliminary form (Schlumpf et al., 1992a).

Materials and Methods

Housing and Breeding Conditions

Forty-nine Long Evans hooded rat fetuses from eight time-pregnant females were used for the study of the prenatal phase. At least six fetuses from each group were examined. The day following insemination was designated gestational day 1 (GD1). Nine Long-Evans rat pups and six Wistar rat pups were used for the postnatal phase. Housing and breeding conditions were as described before (Van Eden and Uylings, 1985; Naeff et al., 1992). The animals were sacrificed by decapitation on postnatal day 1 (PD1; day of birth), PD8, PD15, and PD25. For every age studied, at least two animals were used. No significant differences were observed between the two rat strains as far as the developmental pattern of $GABA_A$ receptors was concerned. The brains were removed and immediately frozen in liquid nitrogen-chilled isopentane. Cryostat sections were made (10 μm for prenatal, 20 μm for postnatal stages), thawed on the slides, and kept at −20°C until fixation. The sections were fixed in a 4% formaldehyde solution in phosphate-buffered saline (PBS), washed in three steps of PBS, dipped in bidistilled water, and dehydrated in grading series of alcohol. After drying, the sections were stored at −80°C for <2 weeks.

In situ *Hybridization*

Three different cDNA oligonucleotide probes (60-mer) were used. These probes are complementary to a subunit-specific region of the intracellular domain between transmembrane regions III and IV (for details, see Malherbe et al., 1990; Schlumpf et al., 1994). Message-sense probes complementary

to the α_1, β_2, and γ_2 subunits, and hybridizations of the antisense probes in the presence of a 100-fold excess of the unlabeled antisense probe were applied to function as controls for specificity. For *in situ* hybridization histochemistry purposes all probes were labeled to a specific activity of 7×10^6 cpm/pg on the 3′ end, using terminal deoxynucleotidyl transferase (GIBCO/Bethesda Research Labs) and ^{35}S-deoxyadenosine-5′-(α-thio)triphosphate (New England Nuclear/DuPont). Autoradiograms of sense-probe labelings are shown in Schlumpf et al. (1994) and in Figure 6 of the present article.

In situ hybridization was performed as previously described (Malherbe et al., 1990). Briefly, triplicate sections from each brain were hybridized with each probe and processed simultaneously. Every fourth section was stained with thionin for optimum tissue structure. Every 16 sections, six consecutive sections were incubated as controls (see above).

Sections were warmed to room temperature and hybridized with 45 μl of hybridization medium containing 300,000–400,000 cpm of ^{35}S-labeled probe. The hybridization medium contained 0.2 gm/ml dextran sulfate, 2× Denhardt's, 8× sodium chloride/sodium citrate solution (SSC; 1× = 165 mM), 0.2 M dithiothreitol, 250 μg/ml tRNA, 250 μg/ml polyA mRNA, 250 μg/ml denatured herring sperm DNA, and 50% deionized formamide. To prevent the tissue from drying, the sections were covered with coverslips during the entire incubation period and stored in sealed boxes containing wetted tissue. Hybridization was performed for 16–19 hr at 42°C.

After incubation the coverslip was removed and the slides were washed in 10× SSC at 55°C (2 × 15 min), 5× SSC (2 × 15 min at 55°C and 1 × 15 min at room temperature), dipped in distilled water, and dehydrated in grading series of alcohol. They were subsequently processed for autoradiography.

Autoradiography

Two autoradiographic methods were employed: (1) the slides were pressed against radiosensitive film (β-Max, Amersham) and exposed for a period of 6–20 weeks depending upon the age; (2) after exposure to the β-Max film and immediately following hybridization, selected sections of postnatal tissue were dipped in photoemulsion (Ilford, K5) and exposed for 3–5 months. The autoradiograms were developed in Kodak D19 and stained with either thionin or hematoxylin/eosin. The terminology of cortical areas and criteria for their delineation and for the delineation of layering have been described previously for the prenatal stages by Uylings et al. (1989), and for the postnatal period by Van Eden and Uylings (1985) and Zilles (1985).

Results

Prenatal Phase

The general prenatal developmental patterns of α_1, β_2, and γ_2 subunit mRNA expression within the CNS have been reported previously (Schlumpf et al., 1992a). As a basis for the detailed description of the postnatal laminar development, an outline of prenatal ontogeny, focusing on the expression of mRNAs encoding the three subunits in cerebral cortex, is given in the following.

At *GD12 and GD14,* no specific signal for any of the three subunit mRNAs is detectable in the wall of the developing cortex (Schlumpf et al., 1992a). At *GD16* (Fig. 1*a,c*), β_2 and γ_2 mRNAs are present in cortical areas, with highest concentrations in piriform cortex. In the developing neocortex, expression of the β_2 subunit is highest in the newly formed cortical plate in the ventrolateral part of the cortical wall. The γ_2 subunit is expressed more strongly and extends more dorsally and deeper into the cortical plate. Expression of the α_1 subunit mRNA is virtually absent within the cortical wall. At *GD18* (Fig. 1*b,d*), a first indication of low expression of α_1 subunit mRNA is observed in the marginal zone and in the cortical plate in the ventrolateral region of the cortical wall. The expression of β_2 mRNA is much stronger and extends farther dorsally into the cortical plate in comparison with GD16. The expression of γ_2 remains the strongest signal with the most widespread distribution. The signal is most intense in the outer zone of the cortical plate throughout the hemisphere, including the medial wall. Lower densities of expression are found in deeper layers down to the intermediate zone. All three subunits are also present in the piriform cortex.

At *GD20* (Fig. 2), all three subunit mRNAs are observed throughout the cortical plate from the rostral pole to the hippocampal region; the expression of β_2 mRNA (Fig. 2*b*) is stronger in the frontal cortical region. Labeling remains higher for β_2 and γ_2 (Fig. 2*c*) than for α_1 subunit mRNA (Fig. 2*a*), with a superficial-to-deep gradient that in case of α_1 and β_2 becomes less distinct in the lateral parts of the cortex. One day before birth, at *GD22*, the distribution pattern of expression of the three subunits resembles that found on the first postnatal day. In neocortex, the β_2 signal has become the most intense one, and α_1 has considerably increased in neocortex and exhibits high levels in piriform cortex. The expression of γ_2 resembles that of the β_2 subunit; however, the outer layers of the piriform cortex are more heavily labeled.

Postnatal Development

The autoradiograms that illustrate the labeling patterns of the α_1, β_2, and γ_2 subunit antisense probes at *PD1* (day of birth) are shown in Figure 3. At this stage of development β_2 subunit mRNA is abundantly present in the cortex (Fig. 3*c*). Throughout the frontal cortex, the probe labels the cortical anlage rather homogeneously. Only the dense upper part of the cortical plate contains more label than the remainder of the cortical wall. The subplate layer shows about the same optical density values as the lower part of the cortical plate. mRNA levels for the α_1 (Fig.3*b*) and γ_2 subunits (Fig. 3*d*) are both lower than that for the β_2 subunit. Generally speaking, the distribution patterns for these subunits are similar to those of the β_2 subunit.

At *PD8* all cortical layers of the adult cortex have sufficiently developed to be distinguishable. The autoradiograms show that the expression of the α_1 subunit has increased markedly in comparison with PD1 and has by now reached levels comparable to that of the β_2 subunit. Furthermore, a more differentiated laminar distribution is found for all three subunits in comparison with earlier stages (see Fig. 4). This laminar differentiation, which shows a high degree of overlap for the three subunits, is the most conspicuous in the parietal area (parietal cortical area 1, Par1). In this granular area, the highest densities are found overlying layer IV for all three subunits. Layers II and VIa also show elevated levels of expression for all three subunits, but layer V still has relatively low levels of expression. Compared to the expression in layer IV, the β_2 subunit (Fig. 4*c*) shows a relatively strong expression over layers II and III.

In the agranular medial cortex the laminar distribution is more differentiated for the three subunits. While the γ_2 (Fig. 4*d*) and α_1 (Fig. 4*b*) subunit mRNAs show a relatively high density over the upper cortical layers (layers II, III, and V), the β_2 subunit mRNA shows a different pattern, with a distinctly higher density over layer III and upper layer V than the rest of the cortical wall. These layers are known to receive the thalamic afferents from the mediodorsal nucleus. Layer II contains relatively high concentrations of the β_2 subunit mRNA, too.

In the agranular lateral prefrontal cortex, dorsal to the rhi-

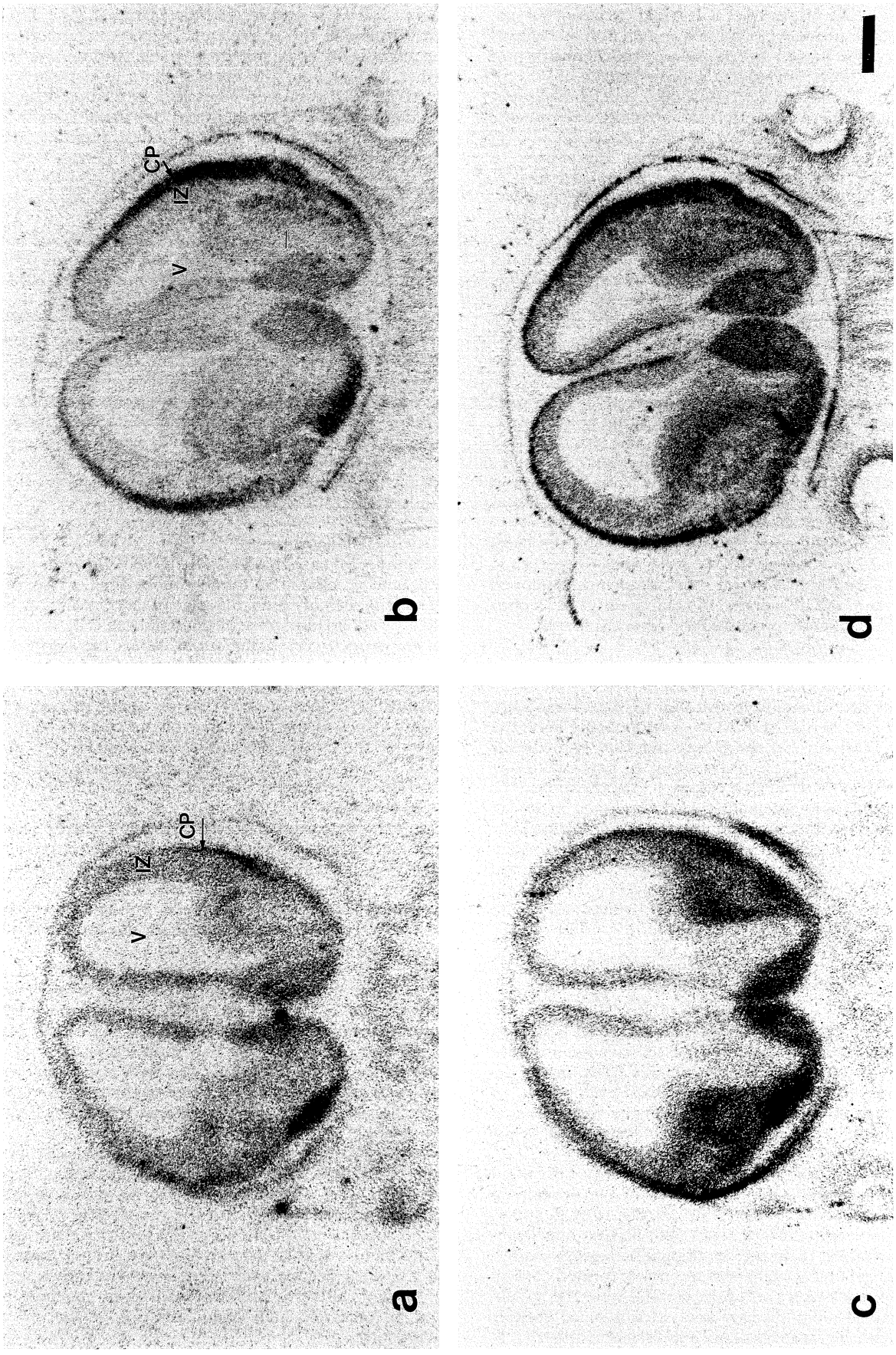

Figure 1. Autoradiograms of transverse sections through frontal telencephalon of rat fetuses of GD16 (*a, c*) and GD18 (*b, d*). *a*, Expression of β_2 subunit mRNA at GD16. *b*, Expression of β_2 subunit mRNA at GD18. *c*, Expression of γ_2 subunit mRNA at GD16. *d*, Expression of γ_2 subunit mRNA at GD18. *CP*, cortical plate; *IZ*, intermediate zone; *V*, ventricle. Scale bar: 475 μm for *a* and *c*, 635 μm for *b* and *d*.

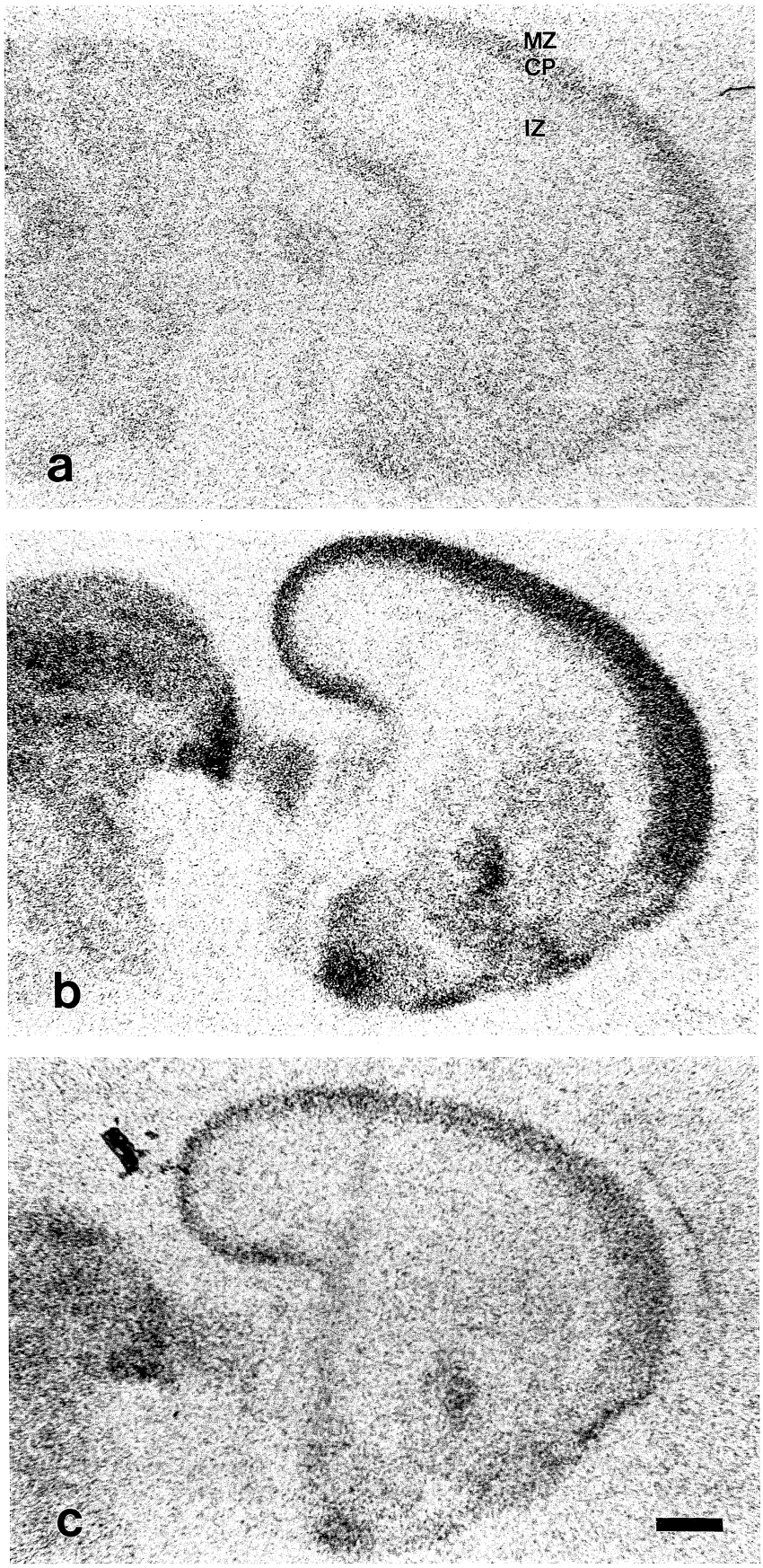

Figure 2. Autoradiograms of sagittal sections of the rostral telencephalon of a rat fetus of GD20. *a,* Expression of the α_1 subunit mRNA. *b,* Expression of the β_2 subunit mRNA. *c,* Expression of the γ_2 subunit mRNA (exposure time was 50% of *a* and *b*). *CP,* cortical plate; *IZ,* intermediate zone; *MZ,* marginal zone. Scale bar, 500 μm.

nal sulcus, a more differentiated distribution is observed for all three subunits. The highest densities are found over layers III and upper layer V, especially for the α_1 and β_2 subunit mRNAs. Only the β_2 signal is also high in the upper layer VIa.

At *PD15* essentially the same laminar distribution patterns were found for the three subunits as at PD8 (see Fig. 5). However, when compared with the patterns of PD8, the expression of the α_1 subunit (Fig. 5*b*) has increased considerably. The amount of α_1 subunit mRNA is by now much higher than that of the β_2 and γ_2 subunits.

In the parietal cortex (Par1), the highest density for all subunits is found in layer IV. Because the expression of the subunits has increased over the layers V and VI, the rest of the cortex is more homogeneously labeled compared with the observations done on PD8. This trend toward a more homogeneous distribution of the expression of the $GABA_A$ subunits continues until PD25.

In the medial (prefrontal) cortex, the distribution of label has also become rather homogeneously distributed in comparison with PD8. The expression of α_1 subunit and β_2 subunit mRNAs (Fig. 5*b*) is highest over layer V, whereas the β_2 (Fig. 5*c*) also shows a peak that corresponds with layer II. The γ_2 is more homogeneously distributed compared to the other two subunits and is only slightly denser over layer V. In the lateral prefrontal cortex (areas AID and AIV; Zilles, 1985), the highest density for all three subunits is found over the superficial layers II, III, and upper V, gradually weakening toward the white matter.

PD25 (Fig. 6) shows essentially the same laminar distribution pattern as PD15, with the exception of the expression of the α_1 subunit (Fig. 6*b*), which has increased further in comparison with the other two subunits.

Cellular Expression

The high-magnification autoradiograms in Figure 7*A–D* show that not all cell types express the $GABA_A$ receptor subunits. From PD8 onward, a differential labeling of different cell types was observed.

Although it is difficult to make an unequivocal classification for every separate cell without using specific cell markers, in most cases it is possible to make a distinction between neuron-like cells and glia-like cells on the basis of morphological criteria (Smart and LeBlond, 1961; Ling et al., 1973; Sturrock, 1976; Vaughan, 1984). In the thionin-stained sections, neuron-like cells are characterized by a pale, spherical nucleus, which often contains a nucleolus, surrounded by a relatively large amount of stained cytoplasm. Most glia-like cell types, like oligodendrocytes, microglia, and neuroglia, have a smaller nucleus that is densely stained. In contrast to the neuron-like cells, the cytoplasm is usually not stained or only a pale rim of cytoplasm is present. Endothelial cells are characterized by a darkly stained elongate nucleus that is often crescent shaped. Given these criteria, the majority of the silver grains were situated above cell somata of neuron-like cells. Endothelial cells were not labeled, nor were most of the glia-like cells. The number of grains overlying the cell soma of neuron-like cells varied considerably. The types of neuron-like cells labeled by the α_1 subunit probe (Fig. 7*A,B*) in the different cortical layers correspond well with those labeled by the β_2 subunit probe (Fig. 7*C,D*). Almost all of the small neuron-like cells of layer IV in the Par1 area are densely labeled. Only few of these cells have a very low number of grains above the somata, which does not significantly reach above background levels (Fig. 7*A*). Layer V shows the greatest difference in labeling between the neuron-like cells (Fig. 7*B–D*). The large neuron-like cells, which in many cases have a triangular- or pear-shaped cell body, contain the densest labeling. The smaller cells in this layer are hardly labeled, or not at all (Fig. 7*B*). This cell class-specific labeling density becomes more pronounced between PD8 and PD15. The γ_2 subunit probe is more homogeneously distributed over the different neuronal cell types.

Discussion

Temporal Pattern of Expression of $GABA_A$ Receptor Subunits

The data presented demonstrate the differential pre- and postnatal distribution of the three subunits that, in combination, are associated with the most common $GABA_A$ receptor in the adult cortex, that is, α_1, β_2, and γ_2 mRNAs. During prenatal development the highest levels of expression are found for the γ_2 subunit, whereas the expression of α_1 is low. Around birth, the ratio of expression of the three subunits changes: the expression of β_2 subunit mRNA, which is moderate between GD16 and GD20, increases strongly, a process that starts in the last few days of gestation. This increase results in a higher expression of this subunit around birth, compared with the other two. The α_1 mRNA begins to rise shortly before birth, and increases at an enhanced rate between PD8 and PD15, when it reaches high degrees of expression.

The low level of expression of the α_1 mRNA appears to be typical of the prenatal period (Laurie et al., 1992; Poulter et al., 1992), and one study even failed to detect this subunit in fetal cortex (Araki et al., 1992). However, it should be noted that the difference is relative rather than absolute, since α_1 mRNA is detected in brain from GD14 and in neocortex from GD18 (Schlumpf et al., 1992a). High levels of expression for other α subunits, namely, α_5 and α_3, are reported during the prenatal period (Araki et al., 1992; Laurie et al., 1992; Poulter et al., 1992). The expression of most of these subunits declines during the neonatal phase, whereas the α_1 subunit expression shows a sharp increase. The temporal pattern of expression of the β_2 and γ_2 subunits described in this article is also in accordance with earlier reports (Gambarana et al., 1991; Zhang et al., 1991; Akari et al., 1992; Laurie et al., 1992). Furthermore, from these studies it is also clear that, as far as the $GABA_A$ receptor is concerned, the adult situation is not yet reached by PD25. The studies of Gambarana et al. (1991) point to a decreasing expression of the α_1 and β_2 subunits between PD30 and PD60, when adult levels of expression are reached.

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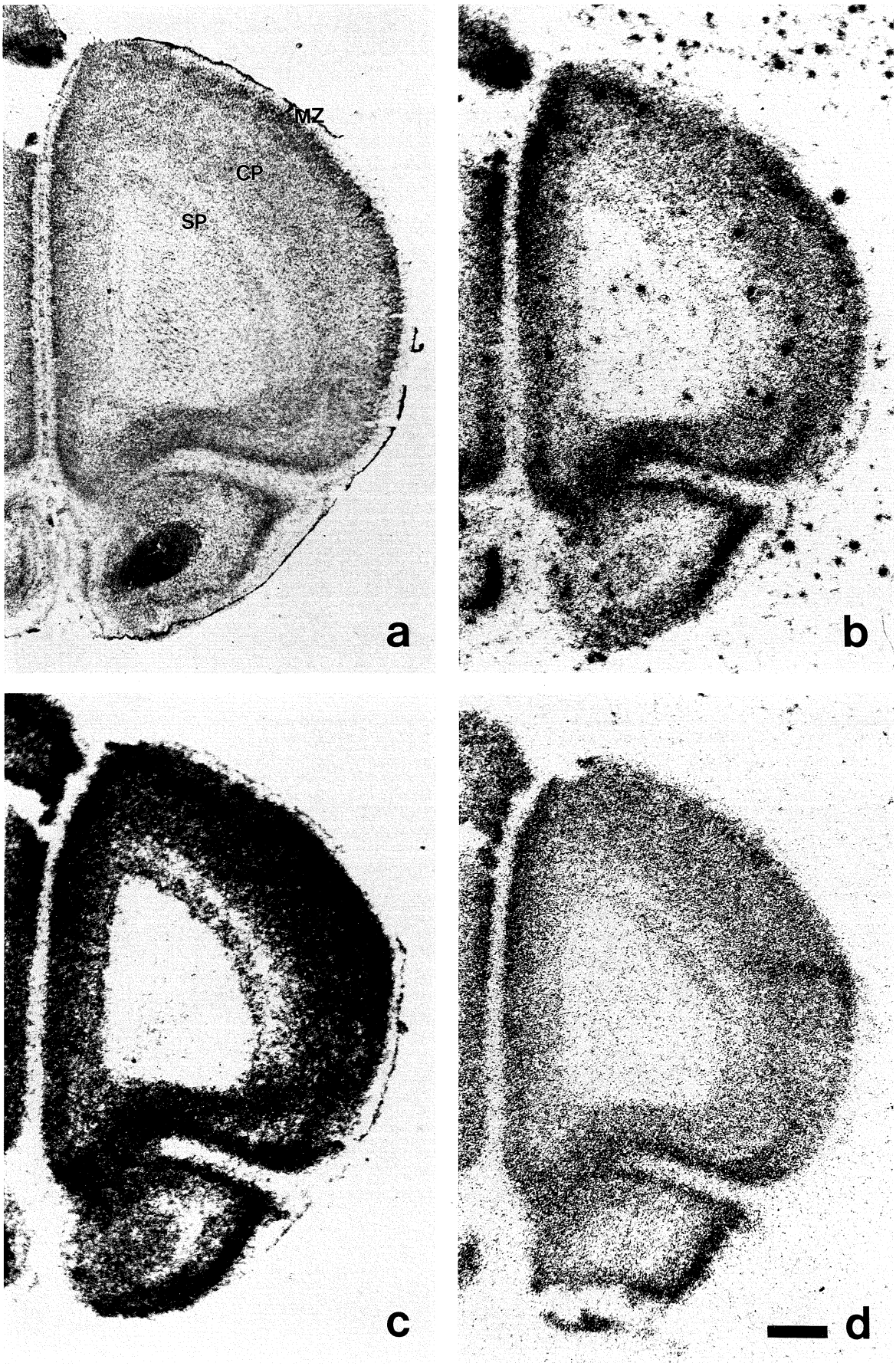
Figure 3. Autoradiograms of transverse sections of the rostral telencephalon of a rat pup at PD1. *a*, Thionin-stained section. *b*, Expression of the α_1 subunit mRNA. *c*, Expression of the β_2 subunit mRNA. *d*, Expression of the γ_2 subunit mRNA. *CP*, cortical plate; *MZ*, marginal zone; *SP*, subplate zone. Scale bar, 500 μm.

Figure 4. Autoradiograms of transverse sections of the rostral telencephalon of a rat pup at PD8. *a*, Thionin-stained section. *b*, Expression of the α_1 subunit mRNA. *c*, Expression of the β_2 subunit mRNA. *d*, Expression of the γ_2 subunit mRNA. *I to VI*, cortical layers I to VI. Scale bar, 750 μm.

Figure 5. Autoradiograms of transverse sections of the rostral telencephalon of a rat pup at PD15. *a*, Thionin-stained section. *b*, Expression of the α_1 subunit mRNA. *c*, Expression of the β_2 subunit mRNA. *d*, Expression of the γ_2 subunit mRNA. *I to VI*, cortical layers I to VI. Scale bar, 900 μm.



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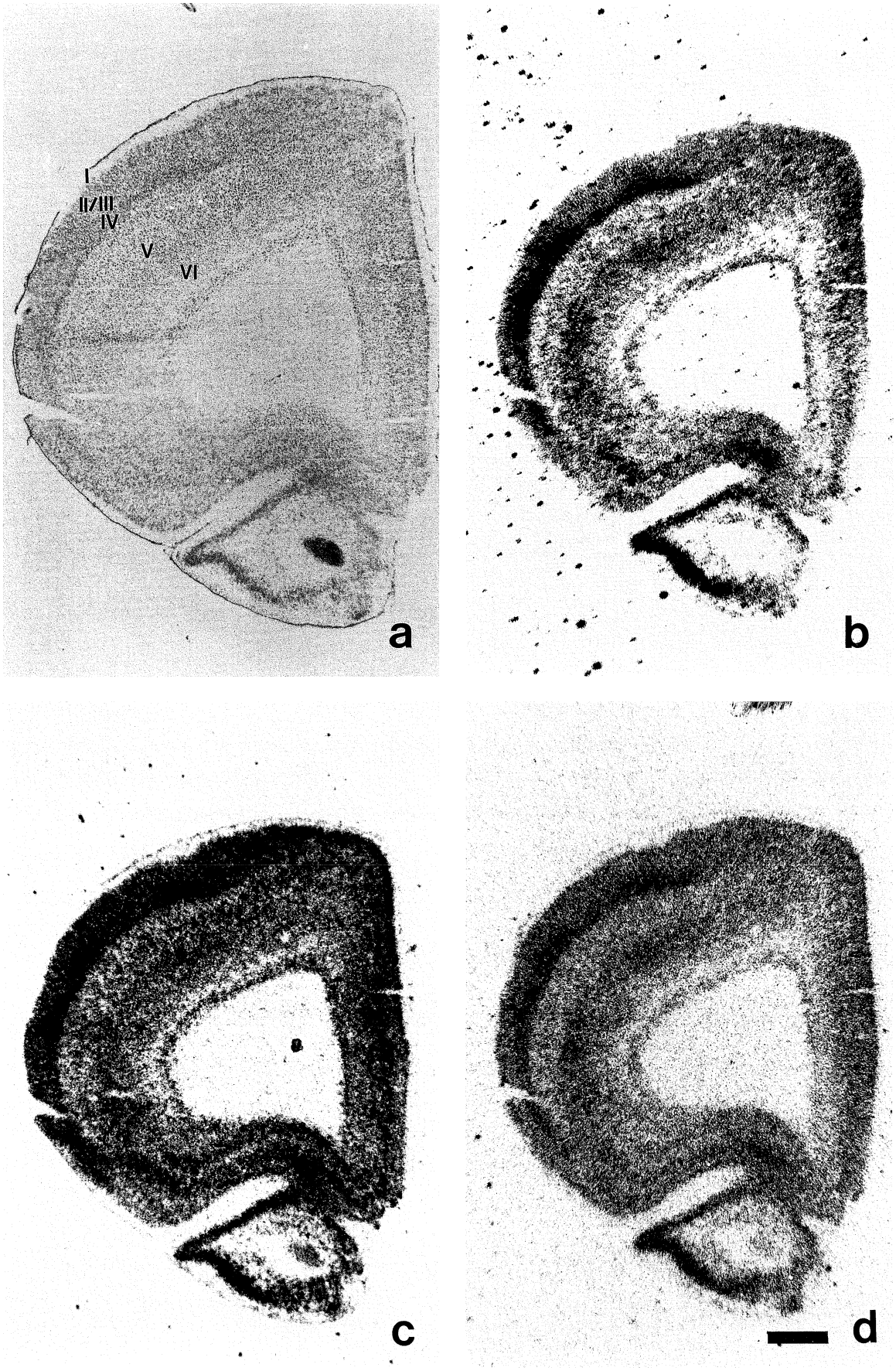

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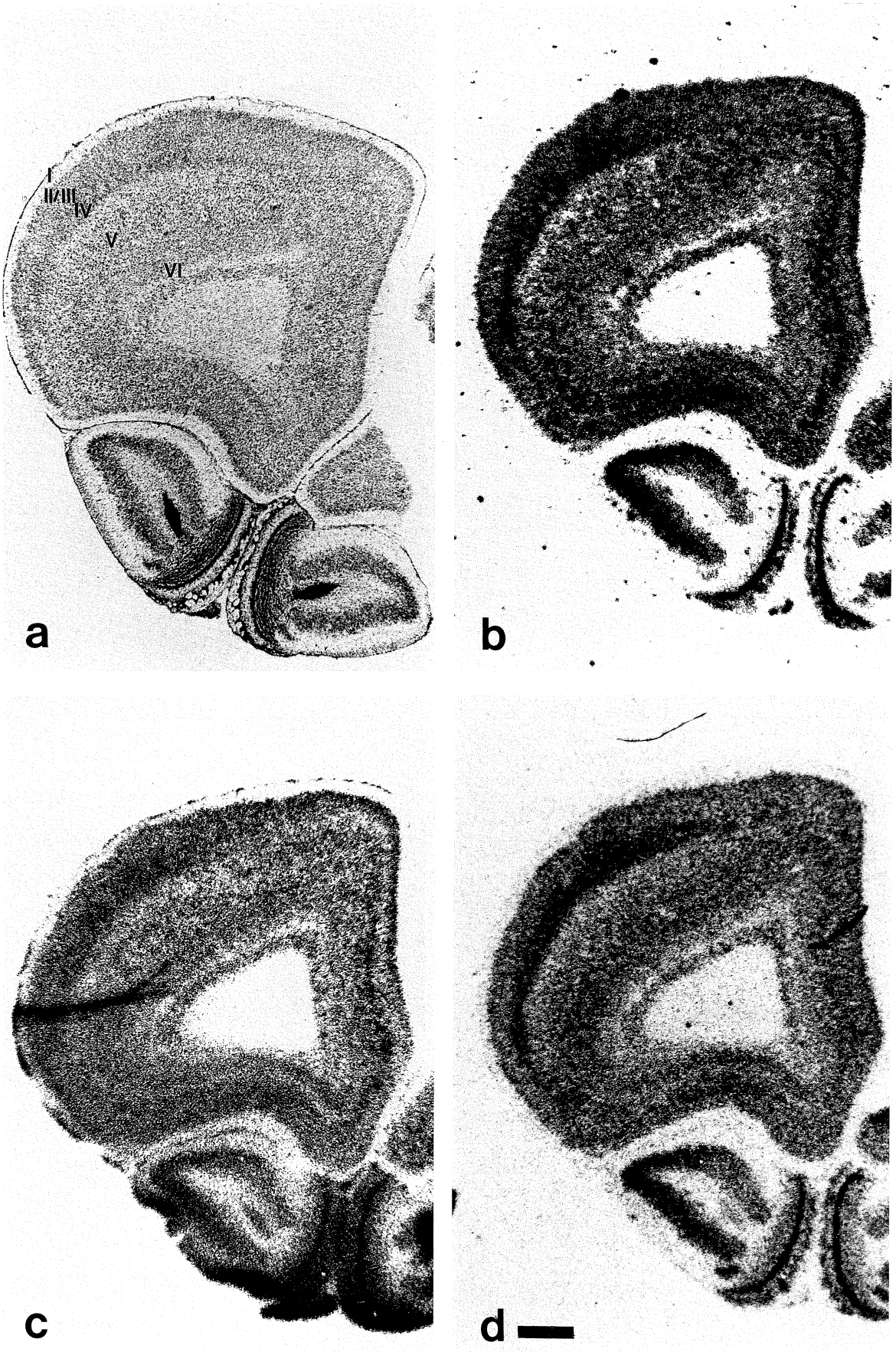

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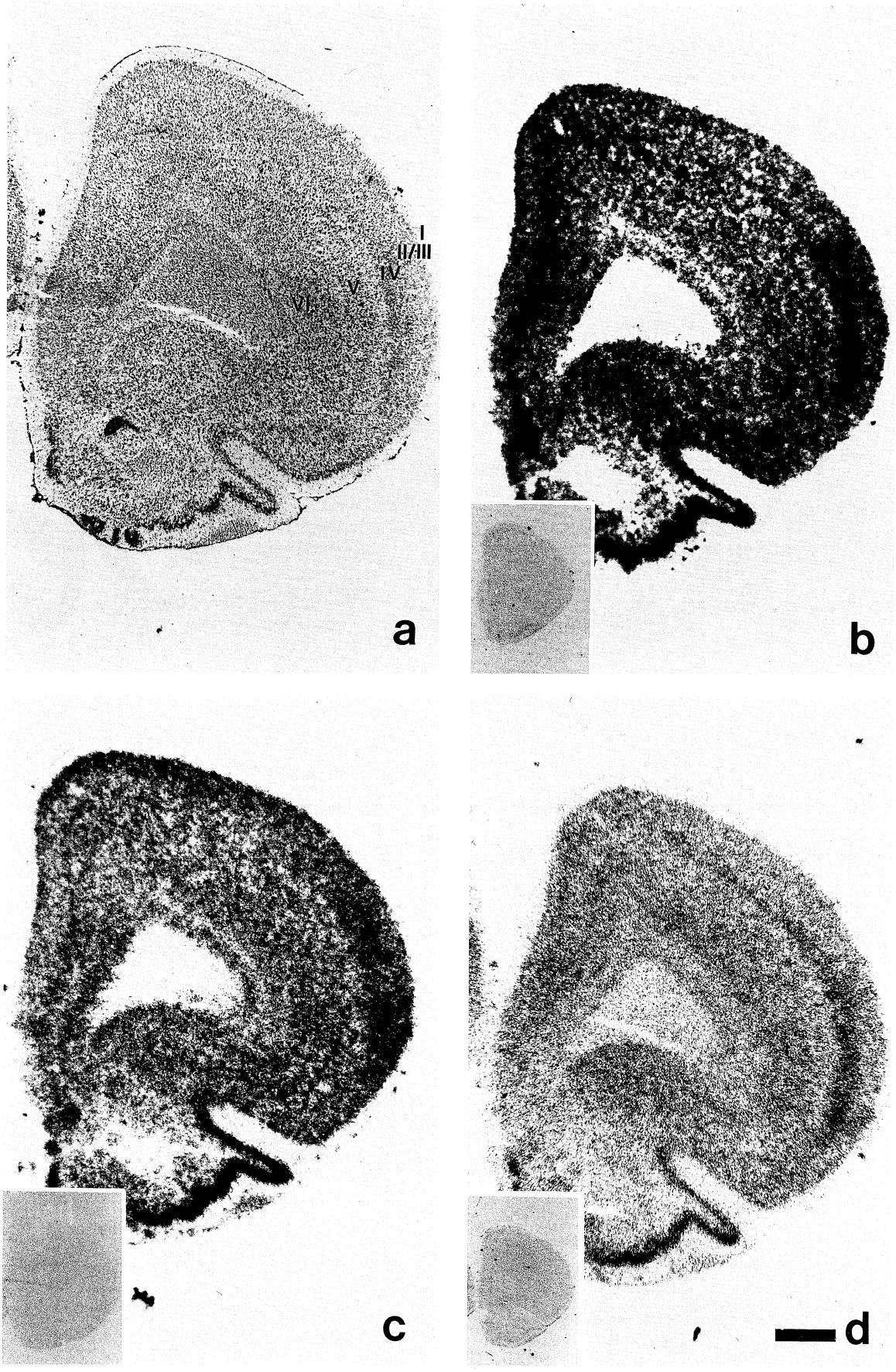

Figure 6.

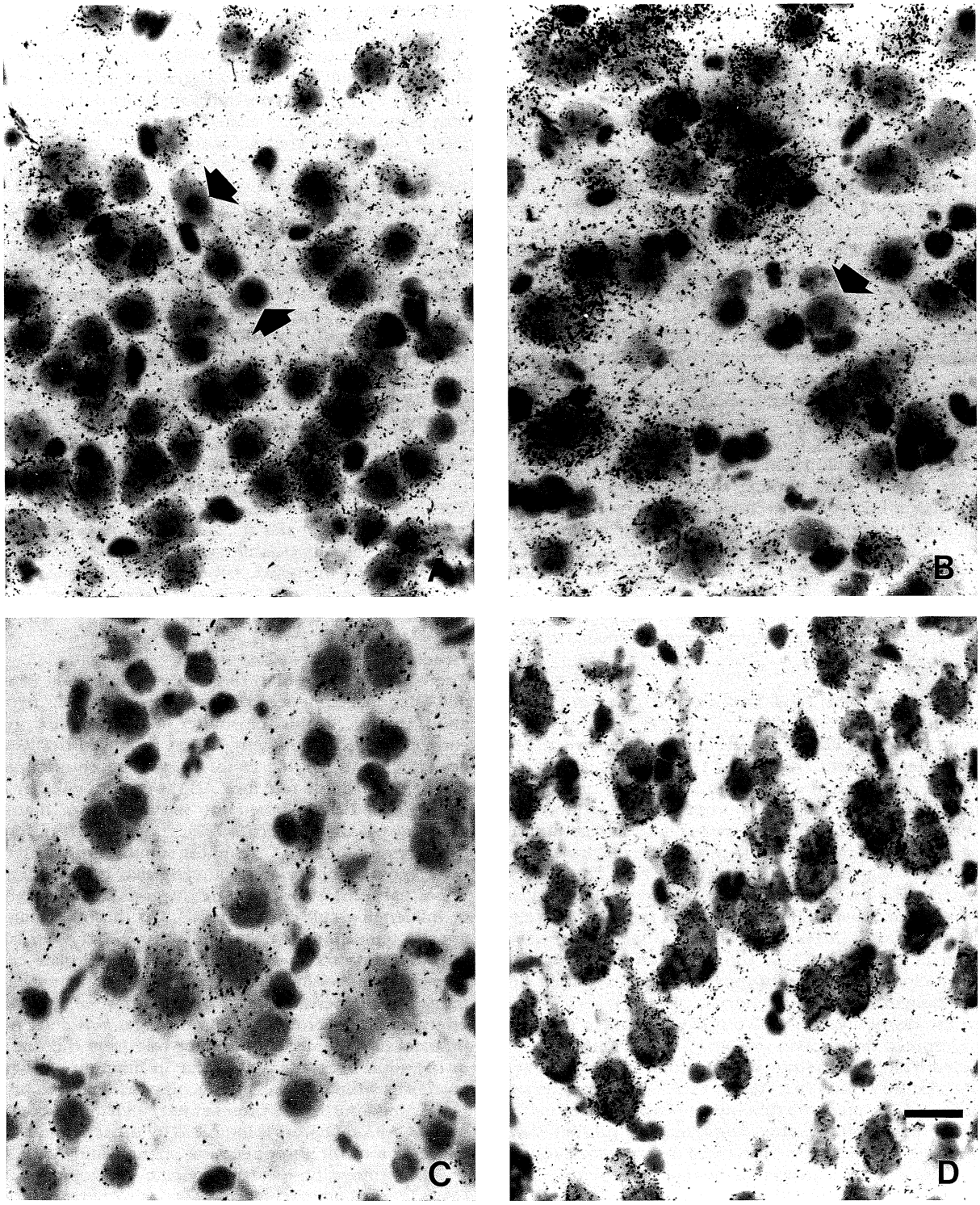

Figure 7. High-power autoradiograms of labeled cells in the neocortex. *A*, Expression of α_1 subunit mRNA in layer IV cells of the Par1 area at PD25. Nearly all neuron-like cells are labeled above background (exceptions marked by *arrows*). Glia-like cells are not frequently labeled. (For criteria of neuron-like and glia-like cells, see text.) *B*, Expression of α_1 subunit mRNA in layer V of the orbital cortex (dorsal part of the agranular insular cortex, AID) at PD25. Note large neuron-like cells are heavily labeled, whereas smaller neuron-like cells (example marked by *arrow*) and glia-like cells are less frequently labeled above background. *C*, Expression β_2 subunit mRNA in layer V of the medial cortex at PD8. Large neuron-like cells labeled above background. *D*, α_1 subunit mRNA in layer V of the medial cortex at PD15. Scale bar: 35 μm for *A, B,* and *D*; 20 μm for *C*.

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Figure 6. Autoradiograms of transverse sections of the rostral telencephalon of a rat pup at PD25. *a*, Thionin-stained section. *b*, Expression of the α_1 subunit mRNA. *c*, Expression of the β_2 subunit mRNA. *d*, Expression of the γ_2 subunit mRNA. *Insets in b–d*, Autoradiograms of neighboring sections hybridized with the respective sense probes. *I to VI*, cortical layers I to VI. Scale bar, 900 μm.

Laminar Distribution in Prenatal Development

As discussed in some detail elsewhere (Schlumpf et al., 1992a), all three subunit mRNAs are first located in the outer zone of the cortical wall at their appearance, γ_2 and β_2 mRNA by GD16, α_1 mRNA by GD18. The signal then spreads toward deeper layers. This means that the subplate remains unlabeled for some days (until GD20). At the stage when the first mRNA signals are detected in cortex (GD16), GABA-immunoreactive cells and fibers are observed in all layers of the cortical wall, with highest density in the marginal zone and subplate layer (Van Eden et al., 1989; Cobas et al., 1991; Meinecke and Rakic, 1992). At the same stage, a low level of BDZ binding is detectable in ventral rostral neocortex; 2 d later (GD18), these receptors are distinctly located in marginal zone and subplate layer (Schlumpf et al., 1983).

There is thus a good degree of correspondence between early stages of GABA immunoreactivity, BZD receptor binding, and $GABA_A$ receptor subunit mRNAs in the outer cortical layers, but less so in deeper layers, especially the subplate layer. Benzodiazepines require the presence of a γ_2 subunit and bind preferentially to $\alpha_1\beta_2\gamma_2$ or $\alpha_3\beta_1\gamma_2$ combinations (Sigel et al., 1990; Verdoorn et al., 1990; Knoflach et al., 1992). When the deeper layer of BZD binding sites becomes distinct at GD18, γ_2 subunit mRNA has extended throughout the cortical plate, but the α_1 and β_2 signals are not yet detectable in deeper layers. It seems possible that the benzodiazepines then bind to a receptor complex composed of different subunits. In principle, $GABA_B$ receptors might serve GABAergic transmission in areas devoid of $GABA_A$ receptors; whether they are present in these zones at early ages presently remains uncertain.

Laminar and Regional Distribution in Postnatal Development

In the adult cortex all classes of neuronal cells receive a substantial GABAergic input (Kisvarday, 1992). The majority of GABAergic terminals in the cortex arise from intrinsic neurons, which form about 20% of the total number of neurons in the cortex (Somogyi, 1990). From what is known about the synaptology and axonal morphology of these intrinsic neurons, it appears that the cortical pyramidal cells are subject to selective inhibition at several levels. However, there is also extensive evidence that GABAergic neurons receive dense axosomatic input from GABAergic terminals as well (for review, see Houser et al., 1984). These terminals can arise from intrinsic local circuit neurons, but they may also have their origin in the subcortical areas (Hendry et al., 1983).

Given the distribution of GABAergic terminals in the cortex, a widespread distribution of $GABA_A$ subunit mRNAs can be expected. The present study, like previous ones (e.g., Araki et al., 1992; Laurie et al., 1992; Poulter et al., 1992), has shown that various $GABA_A$ receptor subunits are expressed in every layer of the developing cortex. However, the present data demonstrate that expression of the $GABA_A$ receptor subunit mRNAs is not simply a matter of maturational status, or a reflection of developmental fluctuations in cell density. The cortical layers are formed according to the "inside-out" rule, meaning that the basal layers mature before the more superficial ones. During prenatal development the highest expression is found in the upper, most immature part of the cortical plate, and in the postnatal period the expression of receptor subunit mRNAs first peaked in layer IV and subsequently increased in more basal layers. The latter increase took place between PD8 and PD25, whereas during the same period the cell density decreases (Van Eden and Uylings, 1985). A similar developmental pattern is also observed in layers II and III. Moreover, at higher magnification it is observed that not all types of cells contribute equally to this increased expression. Especially the large pyramidal neurons in this layer express very high levels of $GABA_A$ receptor subunits mRNAs. In addition, the fact that the older cells of layer V express $GABA_A$ receptor subunit mRNAs at a later point in development than the layer IV cells demonstrates that this developmental pattern is probably more related to maturation of cortical circuitry than it is to the age of the cells.

The observed *regional* differences in the developmental, laminar pattern of expression also point in this direction. In the medial and lateral agranular areas, where layer IV is absent, relatively high labeling densities were first observed over layer III and upper layer V. In adulthood, these layers receive specific thalamic afferents, just like layer IV in the parietal areas (Jones, 1985; Van Eden, 1986). The strong labeling intensity of α_1, β_2, and γ_2 subunit mRNAs above both layers IV (in granular areas) and III/V (in agranular areas) emerges between PD1 and PD8, according to the same schedule despite the differences in maturational status between layer III and layer IV cells. During this period, ingrowth of thalamic fibers into the layers of termination is known to occur in both these areas (Wise and Jones, 1978; Van Eden, 1986). Therefore, expression of $GABA_A$ subunit mRNAs seems to be more related to ingrowth of thalamic afferents than cellular maturation. This joint maturation is further confirmation of the close cooperation between the thalamic afferents and the intrinsic GABAergic circuits (Kisvarday, 1992). Subsequently, between PD8 and PD15, the $GABA_A$ subunits become abundant in layer V pyramidal cells, and these are a major target of the GABAergic internal circuit cells.

Functional Implications

The early presence of GABA and its localization in subplate and marginal zone cells have triggered many hypotheses about the role of this transmitter in the complex process of early cortical development. It was proposed that GABA can act either as a trophic factor or as a neurotransmitter. The notion of a trophic role for GABA is corroborated by *in vitro* studies that demonstrated the propagation of neuritic outgrowth by GABA (Spoerri, 1987). The presently observed early expression of $GABA_A$ receptor subunit mRNA by the relatively most immature cortical cells, that is, those within the upper part of the cortical plate, suggests such a role of GABA in influencing cell differentiation. On the other hand, there are also indications that GABA may act either as an inhibitory transmitter protecting the immature cortical plate cells from overexcitation (Van Eden et al., 1989) or as an excitatory transmitter (Cherubini et al., 1991). Most of these theories imply a transient role of GABA during a particular period of prenatal development, a different role from the one it plays in the adult brain. Such a change in function most probably correlates with a change in the composition of the prominent $GABA_A$ receptor. In the present study a changing composition of the GABA receptors is indicated by the temporally different expression of the three receptor subunit mRNAs.

Furthermore, besides the specific developmental role of GABA during prenatal stages, it has also been demonstrated that, during postnatal development, the pharmacology of receptors changes qualitatively as well as quantitatively (Coyle and Enna, 1976; Kellogg and Pleger, 1989; Daval et al., 1991). For example, GABA is known to potentiate the binding of BDZ ligands. In the neocortex of neonatal rats this feature is very strong and can amount to a 70% increase of flunitrazepam binding in the presence of 10^{-4} M GABA (Daval et al., 1991). In the adult brain, however, GABA can only increase BDZ binding by 20% (Daval et al., 1991). In cortex, this adult feature of GABA receptors is attained between PD10 and PD25, that is, during the same developmental period as high levels of expression were observed of all three (α_1, β_2, and γ_2)

subunit mRNAs of the BZD-sensitive $GABA_A$ receptor typical of adult cortex.

The present data demonstrate the following. (1) $GABA_A$ receptor subunit mRNAs are expressed in the cortex early during prenatal ontogeny and, therefore, functional GABA receptors may be present in the cortical plate shortly after its formation. (2) The three subunits investigated each show a differential developmental pattern, indicating different compositions of cortical $GABA_A$ receptors at different developmental stages. It is most likely that such changes in composition determine functional modifications of the $GABA_A$ receptors in pre- and postnatal development. (3) During the postnatal period, the temporal pattern and laminar differentiation of $GABA_A$ receptor expression of those subunits, which compose the most common receptor of the adult neocortex, concur with the ingrowth of thalamocortical fibers into the granular as well as into agranular areas of the frontal cortex. Thus, the appearance of the $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptor seems to represent a hallmark for the maturation of cortical circuitry.

Notes

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